

Laboratory Experiment Proposal Submission

Experimental Details

Experiment location: E134
Experiment title: Interaction of tubulin with tethered lipid membranes
Experiment date: 7/23/2013
Experiment contactname: David Hoogerheide
Experiment contactphone: 857-928-3232
Experiment contactemail: david.hoogerheide@nist.gov

Chemicals Used

<u>Chemical Name</u>	<u>Health</u>	<u>Flammability</u>	<u>Reactivity</u>	<u>Special Hazards</u>
Sodium Chloride	0	0	0	NONE
Potassium Chloride	2	0	0	
HEPES Buffer	2	1	0	NONE
Sodium PIPES	2	1	0	NONE
Ethanol	2	3	0	NONE
Chloroform	2	0	0	NONE
Sodium Phosphate	0	0	0	NONE
EGTA (ethylene Glycol Tetraacetic Acid)	0	1	0	NONE
EDTA (ethylene Diamine Tetraacetic Acid)	2	0	0	NONE
Sulfuric Acid	3	0	2	COR
Hydrogen Peroxide	3	0	1	OX

Reactants and Resulting Samples

<u>Chemical Name</u>	<u>Hazardous?</u>	<u>Known Hazards</u>
Gold-coated Silicon Wafer With Self-assembled Monolayer	N	

Required Safety Equipment

- ☒ Glove Chemical
- ☒ Glove Cold
- ☒ Glove Neoprene
- ☒ Safety Glasses
- ☒ Hood Acids
- ☒ Hood Organics

Required Laboratory Equipment

- ☒ Balance
- ☒ Hot Plate
- ☒ Ph Meter
- ☒ Lyophilizer
- ☒ Ultrasonic Bath
- ☒ Vacuum Pump

Experimental Write Up

Optimal experimental procedure has not yet been determined, because it is not known what will work. The basic idea is as follows:

1. Lipids (usually precipitated from ~ 1 mL chloroform and lyophilized) are dissolved in the solution of interest by sonication. This solution will generally be a 1 M KCl/ 1 mM HEPES buffer at pH 7, but the pH could vary substantially (4-10) and the concentration as well (perhaps as low as 10 mM). Other buffers of interest are phosphate buffers with NaCl.
2. Bilayers are formed by exposing the self-assembled monolayer (SAM)-coated gold-on-silicon to the lipids.
3. Tubulin is received from the manufacturer as a powder. This is dissolved in a solution of PIPES/MgCl₂/EGTA and stored in very small aliquots (~10-100 uL) of 50 uM solution. These aliquots are flash-frozen in liquid nitrogen (by lowering the tubes into a wide-mouth dewar containing a couple inches (< 1 liter) of liquid nitrogen and then storing them in a -80 C freezer until ready for use.
4. The tubulin solution will then be flowed across the bilayer surface after dilution to about 100 nM.
5. Characterization techniques to be used include neutron reflectivity and electrochemical impedance spectroscopy.
6. Piranha solution may occasionally be used to clean gold surfaces.
7. Self-assembled monolayers are formed from a very dilute ethanol solution of beta-mercaptoethanol and a lipid-like chemical made by Dave Vanderah at IBBR.
8. VDAC procedures are not yet determined.

Experimenter Signature: _____

Date: _____

Lab Responsible Signature: _____

Date: _____